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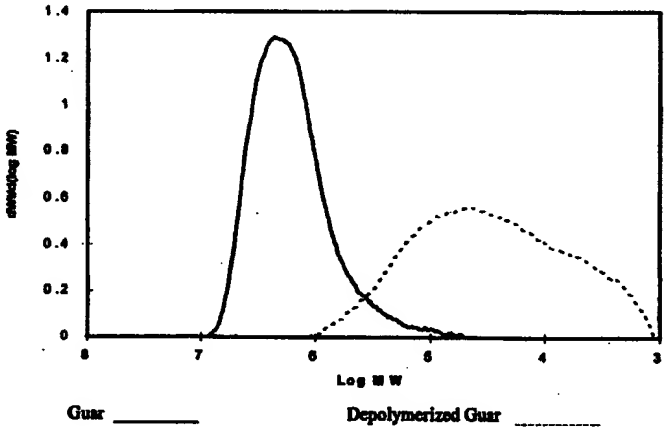
(54) Title: PRODUCTION OF GALACTOMANNAN PRODUCTS BY ENZYMATIC REACTION ON GUAR SPLITS

Guar and Depolymerized Guar from the splits
Molecular Weight by GPC



(57) Abstract

Polygalactomannan products, particularly guar gum products, of greatly reduced molecular weight and viscosity in aqueous solutions thereof, are produced by direct enzymatic depolymerization of polygalactomannan splits.

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PRODUCTION OF GALACTOMANNAN PRODUCTS BY ENZYMATIC REACTION ON GUAR SPLITS

FIELD OF THE INVENTION

This invention relates to a process for the production of low viscosity, low molecular weight galactomannan products by enzymatic reaction on guar splits. More particularly, this invention relates to an improved process for providing free-flowing guar gum powder of low viscosity and low molecular weight by the action of a galactomannan depolymerizing enzyme directly on guar splits.

BACKGROUND OF THE INVENTION

Among the various gum products used as thickeners in the food, pharmaceutical, coatings, mining, oil field, paint, textile, paper and personal care products industries, the most significant has been guar gum. Also guar gum has been recognized as a highly beneficial source of dietary fiber with beneficial effects on serum lipid levels, gastrointestinal travel time and glucose tolerance.

Guar gum is derived from the seed of the guar plant, Cyamopsis tetragonolobus, a pod-bearing nitrogen-fixing legume. Guar gum is a source of polygalactomannan which is a polysaccharide composed primarily of galactose and mannose units. Guar gum is primarily a galactomannan which is essentially a straight chain of D-mannose with single membered D-galactose branches. The mannose units are linked in a 1-4-β-glycosidic linkage with galactose branching occurring by means of a 1-6-linkage on approximately alternate mannose units. Thus, the ratio of galactose to mannose in guar polymer is about 1 to 2.

During processing, the coat of the guar gum seed and the germ portions are generally removed by heating and mechanical

separation, such as by milling, to provide guar splits having a particle size range of generally from about 4 to 20 mesh, U.S. Standard Sieve Series. The endosperm, comprising approximately 40% of the seed and being the galactomannan source, is then hydrated, ground and dried by various processes to produce a guar gum powder having a particle size of generally about 100 mesh or less, useful as a thickening agent. The final milled endosperm, used commercially as guar gum, generally contains about 5-15% moisture, 4-7% protein, less than 7% insoluble residue and about 1.0% ash.

While such guar gum powders have found good success as thickening agents in the various aforementioned industries, its use in the food and pharmaceutical industries has met with rather limited success. At least one of the reasons for its limited success in the food and pharmaceutical industries derives from the fact that it provides a highly viscous solution when hydrated in cold water. Guar gum generally has a molecular weight of about 2,000,000 and the viscosity of a 1% solution will generally range from about 2000 to as high as 8000 cps.

Guar gum powder has been derivatized, such as with propylene oxide to form hydroxypropyl derivatives and the like, to enhance its solubilization properties. Also, guar gum has been subjected to depolymerization action to reduce its viscosity and molecular weight and enhance it solubilization attributes. For the most part such depolymerization reactions on guar gum have occurred by the action of chemical agents on guar powder. Such a process is described, for example, in Japanese Patent Publication 03-290196. The chemical agents employed have generally been either acids or chemical oxidizing agents employed in either solid phase reaction with the guar powder or in solutions of the powder in organic solvents, such as alcohols. However, depolymerized guar powder obtained in these processes generally have increased ash content rendering the product less suitable for use in the food and pharmaceutical fields. Moreover, the need for removal of organic solvents undesirably increases the cost of producing the product.

It has also been suggested in Japanese Patent Publication 01-020063 to dissolve the guar powder in hydrochloric acid and then add an enzyme to produce a guar gum hydrolyzate having a viscosity of about 150-20,000 cps. Also, in Japanese Patent Publication 61-274695 galactose content in guar gum powder is reduced by hydrolyzing an 0.1-10% aqueous solution of the powder with an alpha-galactosidase produced by Mortierella vinacea, after which the resultant product is precipitated in an alcohol and purified. A similar type process is disclosed in Japanese Patent Publication 63-269993.

Each of these processes has various drawbacks, including but not limited to, the increased ash content of the product, the extra process steps required, the undesirable presence of an organic solvent and the need for its removal, and the fact that the enzymatic depolymerization reaction can only be conducted on relatively low guar concentrations, generally less than 10% and usually at a concentration of only about 1% by weight guar gum. Moreover, the enzymatic reaction has required two water additions and two water removal steps.

It has also been disclosed in U.S. Patent 4,693,982 to enzymatically treat guar gum to produce very viscous solutions thereof of relatively low content of insoluble residues. These very viscous aqueous solutions or dispersions of low insoluble residue content are used in high pressure formation fracturing during oil recovery operations. The enzyme employed in the process of the patent, namely Alcalase, removes insolubles but does not depolymerize the guar gum powder since it was desired to maintain the very high viscosity for the intended use of the aqueous solution of the guar gum powder.

Therefore, there is a need for a process for providing depolymerized guar gum having a greatly reduced viscosity and low molecular weight wherein the process does not increase the ash content of the product and does not require the use of acids or chemical oxidizing agents. A further need is for a process which is much simpler to accomplish and is more cost effective. Yet another need is for a process which does not require a multiplicity

of steps or the need to remove process components such as water, organic solvents and the like. A further need is for a process in which much higher concentrations of guar can be employed in the process to make the process more efficient and cost effective.

BRIEF SUMMARY OF THE INVENTION

In accordance with the present invention, it has been discovered that polygalactomannans from guar gum can be depolymerized to provide a polygalactomannan product of low molecular weight and aqueous solutions thereof of greatly reduced viscosity in a process wherein there is a direct reaction of depolymerizing enzyme on guar splits. While the process has been discovered in connection with polygalactomannans from guar gum, it is also applicable to producing depolymerized polygalactomannans from other seed gum sources of polygalactomannans, such as, for example, locust bean, honey locust, tara and flame tree gum and the like. While the process of this invention is applicable to polygalactomannans from any suitable seed gum, for purposes of illustration, it will be described in connection with guar gum.

In accordance with the process of this invention, a galactomannan depolymerizing enzyme is employed to act directly on guar splits to depolymerize the polygalactomannan in the guar splits thereby producing a low molecular guar product capable of producing an aqueous solution thereof of greatly reduced viscosity.

The ability of galactomannase depolymerizing enzyme to react directly on guar splits to produce the product of low molecular weight, greatly reduced viscosity in aqueous solution and relatively unchanged ash content is quite surprising. It was considered necessary to first hydrate the polysaccharide of the endosperm and then reduce it to a fine powder to provide sufficient surface area of polygalactomannan for the depolymerization enzyme to be able to work effectively on the polysaccharide. It is further surprising that the reaction of the polygalactomannan depolymerizing enzyme on the guar splits does not require any increased level of enzyme despite the lower surface area of the

guar splits compared to the powdered guar gums previously subjected to enzymatic reaction.

The depolymerization process of this invention enables one to provide a guar gum product of significantly reduced molecular weight. The molecular weight can be reduced significantly, even up to about 99%, e.g. from about 2,000,000 for guar splits down to about 20,000, generally to a molecular weight of from about 20,000 to 1,000,000, for the depolymerized product. The depolymerization process of this invention also enables one to provide a guar gum product having a greatly reduced viscosity in aqueous solution, e.g. reduced to a viscosity of about 2,000 cps or less, and could even be reduced to a viscosity of about the viscosity of water, i.e. to a viscosity of about 1 cp or less for a 1% aqueous solution measured at room temperature.

In the process according to this invention, the galactomannan depolymerizing enzyme is preferably added to the water to be employed in hydration of the guar splits thereby enabling depolymerized guar powder to be made directly, in a manner which reduces the energy required to accomplish the process and reduces the processing cost by eliminating the cost of first producing guar gum powder. Also, this procedure reduces the need for redissolving guar gum powder in water to carry out depolymerization and then the need to extract the water again. Another significant advantage obtained with the process of this invention is the reduced amount of large size particles obtained on grinding the enzyme treated product. A still further advantage of this invention is the ability to conduct the enzymatic depolymerization reaction at much higher guar concentration than heretofore possible.

The process of this invention permits the obtention of depolymerized guar gum powder directly from the guar splits. This avoids the necessity of first producing guar gum powder which must then be dissolved in water to accomplish the enzymatic reaction and then removal of water again to obtain depolymerized guar gum powder.

The enzymatic depolymerization process of this invention can be carried out by using any suitable lytic enzyme that transforms high molecular weight polysaccharides to lower molecular weight products. Among the suitable galactomannan depolymerizing enzymes that could be employed, there may be mentioned, for example, cellulase, hemicellulase, mannanase, galactomannanase, mannosidase, pectinase, glucanase and the like, and even some enzymes which are commonly known as protease enzymes. The suitable enzymes can be obtained from plants, animals or microrganisms, such as bacterial or fungal sources. As examples of microbial sources of such enzymes, there may be mentioned, for example, Aspergillus species, Aspergillus niger, Aspergillus oxyzae, Trichoderma species, Trichoderma reesei, Bacillus species, Bacillus subtilis, Bacillus lichenformis, Bacillus polymyxa, Rhizopus species and the like, and mixtures thereof.

The enzymatic depolymerization reaction is preferably conducted in an aqueous matrix employed to hydrate the guar splits. The enzymatic depolymerization reaction will generally be conducted in such aqueous matrix wherein the guar concentration can range up to as high as about 50 to 60%, generally from about 20 to 50%, and most preferably from 30 to 40% by weight. The reaction is generally conducted at a temperature of up to about 100°C, preferably up to about 85°C, and more preferably at a temperature between about 65°C and 85°C. The reaction time can vary from a period of about 1 minute to about 1 hour or more. After the depolymerization reaction occurs in the heated aqueous solution, the reaction product is permitted to cool to about room temperature and ground and dried in a suitable mill and drier. For example, the grinding can be accomplished by conventional milling or by flaking.

Any suitable guar splits can be employed in the enzymatic depolymerization process of this invention, including "purified splits", "double purified splits" or "triple purified splits" depending upon the degree of purification. These splits are obtained by mechanical separation of the endosperm from the hull and germ of the guar seed in as pure and intact a form as possible with no other processing steps, as commonly known in the art. In accordance with the present invention, the use of double purified

splits or triple purified splits are particularly preferred.

The products of this invention can be employed in any use where guar gum of reduced molecular weight and viscosity are desired, particularly in oil field, personal care, food and pharmaceutical uses. In food applications, these products could be used as a source of dietary fiber, products for the reduction of blood cholesterol and products for controlling glucose absorption.

BRIEF DESCRIPTION OF THE DRAWING

The drawing Figure is a graph of molecular weight determined by gel permeation chromatography of the product obtained from enzymatic depolymerized guar splits according to this invention and of product from guar splits alone.

DETAILED DESCRIPTION OF THE INVENTION

The invention is illustrated by the following illustrative, but non-limiting examples.

EXAMPLE 1

Into each of two separate 16 ounce glass jars with lids, 100 g water was placed. To one jar, 1 ml hemicellulase enzyme from Aspergillus niger ("Gamanase 1.5L" from Novo) was added. Double purified guar splits in the amount of 100 g was added to each jar. The lids were closed and the jars kept at room temperature with occasional shaking for about 15 minutes. The jars were then placed in a water bath maintained at 65°C for about 30 minutes. The temperature of the water bath was raised to about 85°C and kept at 85°C for another 10 minutes with occasional shaking of the jars during the entire period. The jars were then removed from the heated water bath and permitted to cool to room temperature. The splits were then ground in an Alpine Mill and dried in a fluid bed

drier. The reaction product materials were then sieved through Tyler Mesh screens. The different factions obtained are reported in Table 1.

TABLE 1

	Percent (%) of Product	
Product Size	Split only	Split and Enzyme
+20 mesh	21%	2%
20 to 100 mesh	78%	91%
-100 mesh	1%	7%

The enzyme depolymerized product has less larger sized particles than the milled guar splits.

EXAMPLE 2

The 20 to 100 mesh portion of powders from Example 1 were hydrated in water to make a 1% aqueous solution. The viscosity of the solutions were measured by a Brookfield LVDV instrument at 30 rpm and room temperature. The viscosity measurements of the two solutions are reported in Table 2.

TABLE 2

	Viscosity of Product	
Time	Split only	Split and Enzyme
After 2 hours	95 cp	approx. 1 cp
After 24 hours	216 cp	approx. 1 cp

The 1% solution from the enzyme depolymerized guar splits has a greatly reduced viscosity, i.e. a viscosity similar to water of approximately 1 cp.

EXAMPLE 3

The products obtained in Example 1 were subjected to gel permeation chromatography. The chromatography of guar product obtained from the split and from the depolymerized guar obtained from the enzyme and splits are shown in FIG. 1. The molecular weight of the product from enzyme depolymerized guar splits is greatly reduced from that of the product from guar splits alone.

EXAMPLE 4

The enzyme depolymerization process as described in Example 1 was repeated using 0.1 and 0.5 ml of the hemicellulase enzyme instead of the 1 ml used in Example 1. The 20 to 100 mesh product were used to make 5% aqueous solutions. The solutions were hydrated overnight and viscosity measured by the Brookfield LVDV instrument at 30 rpm and room temperature. The results are reported in Table 3.

TABLE 3

Solution made from	Viscosity, cps
Split + 0.1 ml enzyme	37
Split + 0.5 ml enzyme	9

The viscosity measurement indicates the substantial reduction in viscosity obtained with the enzymatic depolymerization reaction of this invention, even at low levels of enzyme.

The enzymatically depolymerized guar gum product produced according to the process of this invention is readily derivatized by carboxymethylation, hydroxyethylation, hydroxypropylation, cyanoethylation, cationization and the like. Alternatively, the enzymatic depolymerization can be conducted on derivatized guar splits.

With the foregoing description of the invention, those skilled in the art will appreciate that modifications may be made to the invention without departing from the spirit thereof. Therefore, it is not intended that the scope of the invention be limited to the specific embodiments illustrated and described.

C L A I M S:

1. A process for producing a polygalactomannan of reduced viscosity and reduced molecular weight by enzymatic depolymerization, the process comprising the steps of:

(a) providing polygalactomannan splits;

(b) treating the polygalactomannan splits in a reaction mixture with a galactomannan depolymerizing enzyme to produce depolymerization of the polygalactomannan and thereby reducing the molecular weight thereof and the viscosity of an aqueous solution thereof; and

(c) recovering the depolymerized polygalactomannan.

2. The process of Claim 1 wherein the polygalactomannan splits are splits of a leguminous seed selected from the group consisting of guar, locust bean, honey locust, tara and flame tree.

3. The process of Claim 1 wherein the splits are guar splits.

4. The process of Claim 1 wherein the depolymerizing enzyme is an enzyme derived from the group consisting Aspergillus species, Trichoderma species, Bacillus species and Rhizopus species and mixtures thereof.

5. The process according to Claim 1 wherein the galactomannan depolymerizing enzyme is selected from cellulase, hemicellulase, mannanase, galactomannase, mannosidase, pectinase and glucanase and mixtures thereof.

6. The process according to Claim 4 wherein the galactomannan depolymerizing enzyme is selected from cellulase, hemicellulase, mannanase, galactomannase, mannosidase, pectinase and glucanase and mixtures thereof.

7. The process according to Claim 6 wherein the enzyme is hemicellulase.

8. The process according to Claim 7 wherein the enzyme is hemicellulase from Aspergillus niger.

9. The process according to Claim 3 wherein the enzymatic depolymerization process reduces the molecular weight of the polygalactomannan to a range of from about 20,000 to about 1,000,000.

10. The process according to Claim 9 wherein the enzymatic depolymerization process provides a polygalactomannan product having a viscosity of a 1% aqueous solution thereof of about 2,000 cps or less.

11. The process according to Claim 9 wherein the enzymatic depolymerization process provides a polygalactomannan product having a viscosity of a 1% aqueous solution thereof of about the viscosity of water.

12. The process according to Claim 1 wherein the depolymerization enzyme is employed in the reaction mixture in water of hydration for the guar splits.

13. The process according to Claim 12 wherein the guar splits comprises about 20 to about 50% by weight of the reaction mixture.

14. The process according to Claim 3 wherein the depolymerization enzyme is employed in the reaction mixture in water of hydration for the guar splits.

15. The process according to Claim 3 wherein the guar splits comprises about 20 to about 50% by weight of the reaction mixture.

16. The process according to Claim 8 wherein the depolymerization enzyme is employed in the reaction mixture in water of hydration for the guar splits.

17. The process according to Claim 8 wherein the guar splits comprises about 20 to about 50% by weight of the reaction mixture.

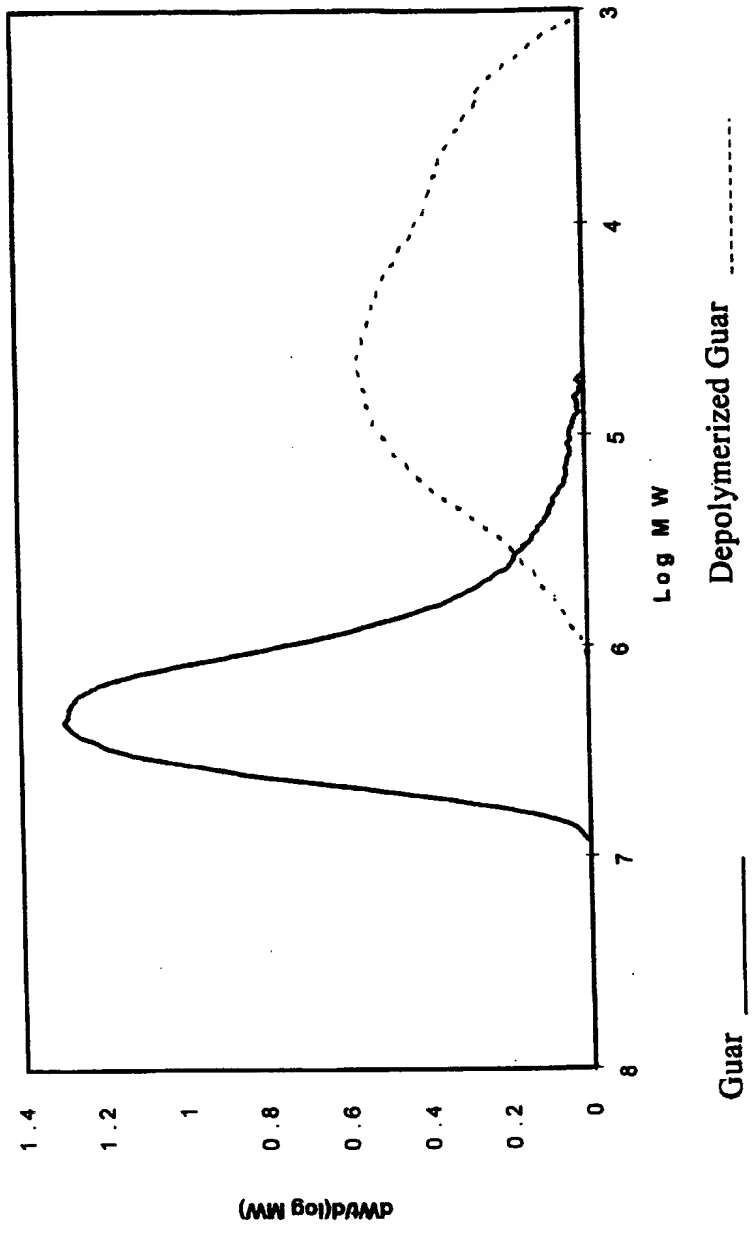
Guar and Depolymerized Guar from the splits
Molecular Weight by GPC
dWWd(log MW)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
3
4
5
6
7
8
Log M W
Guar
Depolymerized Guar

Figure - 1

INTERNATIONAL SEARCH REPORT

I. .national Application No
PCT/US 98/14677

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P19/14 C08B37/14 C12N9/24 C12S3/02 //(C12P19/14, C12R1:685)

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropnate, of the relevant passages	Relevant to claim No.
X	US 4 693 982 A (CARTER WALTER H ET AL) 15 September 1987 cited in the application	1-7, 12-15
A	see abstract see examples 6,7	8-11,16, 17
A	EP 0 121 960 A (UNILEVER NV ;UNILEVER PLC (GB)) 17 October 1984 see abstract see examples 3-6 see examples 10.11	1-17
A	US 5 472 952 A (SMIDT CARSTEN R ET AL) 5 December 1995 see abstract see example 3	1-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of theinternational search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 457 098 A (NAT STARCH CHEM INVEST) 21 November 1991 see abstract see example 3 ---	1-17
A	EP 0 602 991 A (CPC INTERNATIONAL INC) 22 June 1994 see page 4, line 17 - line 24 see page 10, line 1 - line 10 ---	1-17
A	CHEETHAM P S J & UNDERWOOD D R: "Kinetic studies on the enzyme debranching of guar gum" BIOCATALYSIS, vol. 7, 1993, pages 237-247, XP002083906 see abstract see page 238, paragraph 1 ---	1-17
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